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Effects of Smoking and Smoking Cessation on Endothelial Function

1-Year Outcomes From a Randomized Clinical Trial

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Objectives The purpose of this study was to determine whether smoking cessation improves flow-mediated dilation (FMD) of the brachial artery.

Background The long-term effects of continued smoking and smoking cessation on endothelial function have not been described previously.

Methods This was a 1-year, prospective, double-blind, randomized, placebo-controlled clinical trial of the effects of 5 smoking cessation pharmacotherapies. FMD was measured by B-mode ultrasonography before and 1 year after the target smoking cessation date. Cessation was verified by exhaled carbon monoxide levels. ΔFMD was compared among study arms and between subjects who successfully quit smoking and those who continued to smoke. Predictors of baseline FMD and ΔFMD were identified by multivariable regression.

Results The 1,504 current smokers (58% female, 84% white) were 44.7 (SD = 11.1) years of age and smoked 21.4 (SD = 8.9) cigarettes/day. Baseline FMD was similar in each treatment arm ($p = 0.499$) and was predicted by BA diameter ($p < 0.001$), reactive hyperemia blood flow ($p < 0.001$), high-density lipoprotein cholesterol ($p = 0.001$), and carbon monoxide ($p = 0.012$) levels. After 1 year, 36.2% quit smoking. FMD increased by 1% (6.2% [SD = 4.4%] to 7.2% [SD = 4.2%]) after 1 year ($p = 0.005$) in those who quit, but did not change ($p = 0.643$) in those who continued to smoke. Improved FMD among quitters remained significant ($p = 0.010$) after controlling for changes in brachial artery diameter, reactive hyperemia, low-density lipoprotein cholesterol, and the presence of a home smoking ban.

Conclusions Despite weight gain, smoking cessation leads to prolonged improvements in endothelial function, which may mediate part of the reduced cardiovascular disease risk observed after smoking cessation. (Smoking Cessation Medications: Efficacy, Mechanisms and Algorithms; NCT00332644) (J Am Coll Cardiol 2010;55:000–000)

Epidemiological studies have established strong relationships between cigarette smoking, atherosclerosis burden, and cardiovascular disease (CVD) events; approximately one-third of smoking-related premature deaths are due to CVD (1–5). Significant CVD risk reduction and mortality benefits are associated with smoking cessation (2,3,6,7). Cigarette smoking promotes atherogenesis and CVD through multiple interactive mechanisms including vasomotor, neurohormonal, and hematologic dysfunction, increased oxidative stress, and dyslipoproteinemia; however, the exact mechanisms are not fully understood (4,5).

Endothelial dysfunction is an early event in atherogenesis that results in inflammation, vasoconstriction, and thrombosis (8,9). It has been hypothesized that endothelial cell damage from inhaled cigarette smoke contributes to vascular injury, atherogenesis, and increased CVD risk; smoking as few as 2 cigarettes/day doubles the number of nuclear-damaged endothelial cells in the circulating blood (4,5,8,10–12). Flow-mediated vasodilation (FMD) of the

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Abbreviations and Acronyms

BA = brachial artery
BP = blood pressure
CO = carbon monoxide
CVD = cardiovascular disease
FMD = flow-mediated dilation
HDL-C = high-density lipoprotein cholesterol
hsCRP = high-sensitivity C-reactive protein
LDL-C = low-density lipoprotein cholesterol
RH = reactive hyperemia

brachial artery (BA) is a noninvasive, validated measure that quantifies endothelial function and predicts future CVD events (13,14). In clinical and epidemiological studies, smoking-induced endothelial dysfunction seems to be dose related and may be reversible after smoking cessation (11,12,15). However, the mechanisms by which smoking cessation reduces CVD risk are unclear, and the long-term effects of continued smoking and smoking cessation on endothelial function have not been established. This study evaluated the effects of current smoking and smoking cessation on endothelial function in a prospective, randomized clinical trial of smoking cessation pharmacotherapy (16). We hypothesized that smoking cessation would improve endothelial function of the BA.

Methods

Study participants and design. The institutional review board at the University of Wisconsin School of Medicine and Public Health approved this study. All subjects provided written informed consent. This was a 3-year longitudinal, randomized, double-blind, placebo-controlled trial to evaluate the efficacy of 5 smoking cessation pharmacotherapies and to examine the natural history of continued smoking and smoking cessation on subclinical atherosclerosis (16). This article describes the independent predictors of BA FMD in current smokers at baseline, before smoking cessation treatment, and 1 year after the target quit date.

Participants were randomized to 1 of 6 treatment conditions: nicotine lozenge, nicotine patch, sustained-release bupropion, nicotine patch plus nicotine lozenge, sustained-release bupropion plus nicotine lozenge, or placebo (Online Appendix 1) (16). All participants received individual counseling sessions (16). Major inclusion criteria were age 18 years and older, smoking ≥10 cigarettes/day, expired carbon monoxide (CO) level >9 ppm, and stated motivation to quit smoking. Major exclusion criteria were blood pressure (BP) >160/100 mm Hg, myocardial infarction within the previous 4 weeks, heavy alcohol use, history of seizure or serious head injury, use of contraindicated medications, and current pregnancy or breastfeeding (16).

Study procedures. Subjects were recruited from communities in and around Madison and Milwaukee, Wisconsin, from January 2005 to June 2007. The baseline clinical trial visit included measurement of anthropometric data, fasting laboratory tests, and completion of validated questionnaires and interviews. Physical activity was assessed by the International Physical Activity Questionnaire (17). Smoking burden was evaluated by current cigarette smoking (cigarettes/day) and pack-years (current cigarettes per day × years smoked). Recent smoke exposure was measured by an exhaled CO level, which reflects smoking efficiency and recent smoke exposure. Smoking status was assessed by self-reported 7-day point-prevalence abstinence and was confirmed by an expired CO level of <10 ppm. Cotinine was not used to assess abstinence because interventions in this study included nicotine replacement therapy, which could have produced false-positive results. When used with self-report to indicate whether a person has smoked, CO and cotinine levels show high agreement (18). Three self-reported measures of environmental smoke exposure were evaluated at baseline: whether smoking was allowed inside the home (home smoking ban), whether the subject lives with a partner/spouse who smokes, and whether smoking was allowed in the workplace. There is a strong relationship between self-reports of home and work smoke exposure and cotinine levels (19). Fasting blood samples were obtained by venipuncture and were refrigerated. Plasma aliquots were isolated by centrifugation and frozen at −70°C.

Measurement of BA reactivity. Endothelial function was evaluated by measuring the FMD of the BA in a core ultrasound laboratory using a standardized protocol (13,20). BA reactivity studies were performed at baseline, before initiating therapy, and 1 year after the target quit date. Subjects were required to be fasting and not use any tobacco-containing products for 8 h before the study. Subjects were placed in a supine position in a temperature-controlled room for 10 min before imaging. A BP cuff was placed on the widest part of the proximal right forearm. Using a 10-MHz linear array vascular ultrasound transducer and a Siemens Medical Solutions (Issaquah, Washington) CV70 ultrasound system, the BA was located above the elbow and scanned in longitudinal sections. Extravascular landmarks were identified and labeled to ensure reproducibility within and between studies. After recording baseline B-mode ultrasound images of the BA and spectral Doppler ultrasound images of flow, the cuff was inflated to 250 mm Hg for 5 min to induce reactive hyperemia (RH). Immediately after deflation, spectral Doppler ultrasound images were obtained to verify hyperemia. BA images were obtained 60 and 90 s later. Studies were recorded digitally; BA diameters were measured in triplicate with a digital border tracing tool (Access Point Web 3.0, Freeland Systems, Westfield, Indiana) by 3 readers blinded to subject information and treatment. The same reader read the baseline and follow-up studies in all subjects. The primary outcome variable was the maximum FMD (in %), the largest percentage of change in the BA diameter after RH relative to the baseline diameter. The absolute maximum FMD (in cm), the absolute difference in BA size after RH compared with baseline, also was reported.

All sonographers completed a standardized certification program. Ultrasound equipment was monitored using a small-parts phantom. In our laboratory, subjects who un-

derwent repeat FMD scans approximately 2 weeks apart had an interscan FMD difference of only 0.26% (−0.43% to +0.72%, p = 0.498); the median inter-reader variability was −0.14% to +0.09%, with correlations of 0.97 to 0.99 (p < 0.001) (20). Re-evaluation of inter- and intrareader variability in 2009 showed similar, small mean FMD differences between the 2 readers evaluated (0.13% to 0.26%).

Data analysis. All analyses were performed with SPSS software (Version 17.0, SPSS, Inc., Chicago, Illinois). Continuous variables are given as mean (SD); categorical variables are presented as percentages. Differences in baseline BA diameter and maximum FMD between the treatment conditions were evaluated using univariate analysis of variance and Tukey's test. Because treatment condition was not significantly related to the dependent variables, it was not covaried in further baseline analyses. Pearson correlations were used to identify univariate predictors of BA diameter and FMD. Partial correlations controlling for BA diameter were obtained for baseline FMD. Using candidate variables (p < 0.10) from partial correlations, multivariate analyses were performed to determine variables that were independently associated with baseline FMD, before the initiation of smoking cessation pharmacotherapy. Because baseline BA diameter was such a strong predictor of baseline FMD, all models were adjusted for BA diameter and reader. Because sex was strongly correlated with baseline diameter, separate analyses were performed for men and women.

The mean difference in the BA diameter (in cm) and the absolute ΔFMD (in %) from baseline to 1 year after the target quit date were analyzed. *t* tests were used to evaluate differences between subjects who did and did not return for the 1-year FMD visit. Next, we conducted a series of univariate analyses of variance to test for differences among the treatment conditions on 2 dependent variables: changes in baseline diameter and maximum FMD from baseline to year 1. Because treatment condition was not significantly related to the dependent variables, it was not covaried in later analyses. The *t* tests were used to evaluate differences in ΔFMD and ΔBA diameter by sex and within and between groups who quit smoking versus those who continued smoking. Multivariate regression analyses were used to determine variables that independently predicted ΔFMD at 1 year after the target quit date. All models were adjusted for ΔBA diameter and reader. Baseline variables and changes in variables from baseline to year 1 were considered in the ΔFMD analysis.

All variables were examined with regard to their distributional properties by visual inspection and assessment of kurtosis and skew. Four variables were not normally distributed: pack-years, triglycerides, high-sensitivity C-reactive protein (hsCRP), and baseline BA flow. These values were log-transformed and comparisons were repeated, but comparable results were seen. Log-transformed values of these variables were retested in the multivariate models, and no major changes were observed. Residual plots from all reported regression models were inspected and seemed to be normally distributed.

Results

Subject characteristics. Subject characteristics at baseline and after 1-year follow-up are shown in Table 1. Baseline studies were performed in 1,504 current smokers before

Table 1 Subject Characteristics at Baseline and 1 Year After the Target Quit Date

		Relapsed			Abstained			p Values	
	All Subjects, Baseline	Baseline, Mean (SD)	1 Year, Mean (SD)	Δ (SD)	Baseline, Mean (SD)	1 Year, Mean (SD)	Δ (SD)	Baseline, Relapsed vs. Abstainer	Δ, Relapsed vs. Abstainer
n	1,504	1,125	589		379	334			
Body mass index (kg/m^2)	29.0 (6.5)	28.9 (6.5)	29.0 (6.4)	0.24 (1.73)	29.0 (6.6)	30.5 (6.7)	1.61 (2.04)	0.842	<0.001
Waist circumference (cm)	95.9 (16.2)	95.5 (15.9)	96.1 (15.8)	0.99 (6.36)	97.1 (16.9)	100.0 (16.0)	3.05 (10.67)	0.103	0.002
CO (ppm)	25.7 (12.4)	26.5 (12.7)	19.1 (12.1)	−7.2 (13.0)	23.6 (11.4)	2.1 (1.9)	−21.3 (11.2)	<0.001	<0.001
Heart rate (beats/min)	62.9 (10.5)	74.2 (9.6)	71.4 (9.8)	−2.0 (10.7)	73.1 (9.6)	69.6 (9.3)	−3.3 (10.7)	0.049	0.089
Systolic BP (mm Hg)	124.9 (14.7)	119.0 (14.5)	116.6 (14.5)	−2.8 (13.5)	120.6 (14.2)	116.5 (14.9)	−4.4 (14.6)	0.066	0.102
Diastolic BP (mm Hg)	74.0 (9.4)	74.1 (10.2)	72.3 (10.1)	−1.6 (9.9)	75.1 (9.5)	73.7 (10.7)	−1.4 (10.4)	0.091	0.744
Total cholesterol (mg/dl)	183.9 (35.4)	183.4 (36.0)	183.6 (37.5)	0.8 (28.8)	185.4 (33.5)	185.8 (34.9)	1.3 (28.2)	0.355	0.273
LDL-C (mg/dl)	118.9 (30.6)	118.2 (31.0)	119.3 (32.3)	0.38 (22.2)	121.0 (29.5)	119.3 (31.2)	−0.81 (24.2)	0.144	0.449
HDL-C (mg/dl)	42.0 (13.5)	42.0 (13.5)	42.7 (14.4)	0.4 (8.7)	41.9 (13.6)	44.9 (13.6)	2.8 (8.3)	0.927	<0.001
Triglycerides (mg/dl)	143.3 (101.7)	144.1 (107.9)	134.5 (100.4)	−10.0 (80.4)	140.9 (80.7)	138.3 (90.4)	−0.1 (75.2)	0.601	0.065
Fasting glucose (mg/dl)	94.9 (17.7)	95.3 (18.7)	97.2 (23.1)	0.9 (16.1)	94.2 (13.7)	98.5 (26.9)	4.3 (22.7)	0.299	0.008
HbA_{1c} (%)	5.6 (0.6)	5.6 (0.7)	5.73 (0.760)	0.12 (0.50)	5.6 (0.5)	5.69 (0.631)	0.14 (0.42)	0.788	0.543
hsCRP (mg/l)	2.11 (11.0)	2.22 (12.6)	3.98 (7.70)	2.06 (7.07)	1.72 (2.94)	3.89 (5.42)	2.22 (5.77)	0.454	0.715
Baseline BA diameter (cm)	0.395 (0.073)	0.393 (0.072)	0.403 (0.072)	0.005 (0.020)	0.402 (0.074)	0.409 (0.074)	0.006 (0.019)	0.054	0.565
Baseline flow (ml/min)	116.8 (76.8)	117.1 (78.5)	127.5 (80.4)	11.4 (70.0)	116.2 (71.4)	135.5 (132.8)	19.6 (128.0)	0.863	0.263
RH blood flow (ml/min)	686.8 (269.3)	679.5 (269.7)	719.5 (263.6)	39.8 (228.8)	708.8 (267.4)	766.9 (295.6)	48.8 (268.4)	0.089	0.626
Absolute FMD (cm)	0.023 (0.015)	0.023 (0.015)	0.025 (0.014)	0.001 (0.015)	0.024 (0.015)	0.028 (0.015)	0.004 (0.017)	0.210	0.017
FMD (%)	6.15 (4.35)	6.48 (4.26)	6.58 (4.05)	0.04 (4.46)	6.15 (4.36)	7.20 (4.18)	1.00 (4.30)	0.391	0.005

BA = brachial artery; BP = blood pressure; CO = carbon monoxide; FMD = flow-mediated dilation; HbA_{1c} = glycosylated hemoglobin; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; RH = reactive hyperemia.

Table 2 Independent Associations With Maximum FMD (%) at Baseline in All Subjects (R^2_{adj} = 0.225)

	Beta	Standardized Beta	95% Confidence Interval		p_{adj}
			Lower Limit	Upper Limit	
Constant	19.107	—	17.228	20.985	<0.001
BA diameter (cm)	−35.049	−0.582	−39.183	−30.915	<0.001
RH blood flow (ml/min)	0.006	0.364	0.005	0.007	<0.001
HDL-C (mg/dl)	−0.030	−0.095	−0.048	−0.012	0.001
CO (ppm)	−0.025	−0.069	−0.044	−0.005	0.012
Reader	−0.585	−0.118	−0.854	−0.317	<0.001

Abbreviations as in Table 1.

initiating therapy. Subjects (58.2% female, 83.9% white, 13.6% African American) were 44.7 (SD = 11.1) years of age and smoked 21.4 (SD = 8.9) cigarettes/day with a smoking burden of 29.4 (SD = 20.4) pack-years. These values did not differ between pharmacotherapy treatment arms. They consumed 16.0 (SD = 23.9) alcohol-containing beverages per month and performed 122.0 (SD = 150.1) MET (metabolic equivalent)-hours/day of moderate-vigorous and 11.1 (SD = 21.4) MET-hours/day of leisure activity. Regarding environmental smoke exposure, 27.9% lived with a spouse/partner who smoked, 46.3% had a home smoking ban, and 51.4% had smoking bans at work. Baseline FMD was 6.2% (SD = 4.4%) and the BA diameter was 0.40 (SD = 0.07) cm. Baseline FMD ($p = 0.297$), BA diameter ($p = 0.424$), BA blood flow ($p = 0.146$), and RH flow ($p = 0.640$) did not differ among pharmacotherapy treatment arms.

Evaluation of baseline FMD and BA diameter. Baseline FMD correlated significantly ($p < 0.001$) with age ($r = -0.14$), systolic BP ($r = -0.11$), diastolic BP ($r = -0.15$), cigarettes smoked per day ($r = -0.09$), current pack-years ($r = -0.13$), creatinine ($r = -0.11$), glucose ($r = -0.07$, $p = 0.017$) and hsCRP ($r = -0.06$, $p = 0.027$). The strongest correlation was between BA diameter and FMD ($r = -0.34$, $p < 0.001$); therefore, partial correlations (adjusting for BA diameter) with baseline FMD were identified and included RH flow ($r_{adj} = 0.343$, $p < 0.001$), cigarettes smoked per day ($r_{adj} = -0.10$, $p = 0.019$), current pack-years ($r_{adj} = -0.12$, $p = 0.005$), hsCRP ($r_{adj} = -0.12$, $p = 0.005$), and high-density lipoprotein cholesterol ([HDL-C], $r_{adj} = -0.16$, $p < 0.001$).

BA diameter ($p < 0.001$), RH flow ($p < 0.001$), CO ($p = 0.012$) and HDL-C ($p = 0.001$) were independently associated with baseline FMD ($R^2_{adj} = 0.225$) (Table 2). A model of baseline FMD without RH flow resulted in the same predictors but with a lower R^2_{adj} (0.142) (Online Appendix 2). Models using absolute FMD (in centimeters) as an outcome variable identified similar predictors, but with a lower R^2_{adj} (0.152). Men had larger BA diameters (0.46 [SD = 0.06] cm vs. 0.35 [SD = 0.05] cm, $p = 0.001$) and waist circumferences (101.1 [SD = 14.6] cm vs. 92.2 [SD = 16.3] cm, $p = 0.002$) than women, as well as lower FMD (5.5% [SD = 3.7%] vs. 6.7% [SD = 4.7%], $p < 0.001$). In men (Table 3), FMD was independently associated with BA diameter ($p < 0.001$) and RH flow ($p < 0.001$), with a trend for triglycerides ($p = 0.058$). In women (Table 4), FMD was independently associated with BA diameter ($p < 0.001$), RH flow ($p < 0.001$), and CO ($p = 0.011$), with a trend for HDL-C ($p = 0.061$).

Because BA diameter was consistently associated with FMD and has been shown to predict CVD events as well as FMD (14,21), associations with BA diameter were identified. Significant ($p < 0.005$) correlations for BA were seen with body mass index ($r = 0.24$), waist circumference ($r = 0.42$), systolic BP, diastolic BP, age, cigarettes smoked per day, pack-years, creatinine, glucose, hematocrit, HDL-C, triglycerides, physical activity, and C-reactive protein ($p = 0.029$). BA diameter (Table 5) was independently associated with male sex ($p < 0.001$), age ($p < 0.001$), waist circumference ($p < 0.001$), glucose ($p = 0.018$), and physical activity ($p = 0.001$).

Table 3 Independent Associations With Maximum FMD (%) in Men at Baseline (R^2_{adj} = 0.237)

	Beta	Standardized Beta	95% Confidence Interval		p_{adj}
			Lower limit	Upper Limit	
Constant	17.789	—	15.104	20.474	<0.001
BA diameter (cm)	−33.226	−0.483	−39.234	−27.219	<0.001
RH blood flow (ml/min)	0.004	0.334	0.003	0.005	<0.001
Triglycerides (mg/dl)	0.002	0.078	0.000	0.005	0.058
Reader	−0.589	−0.132	−0.950	−0.227	0.001

Abbreviations as in Table 1.

Table 4 Independent Associations With Maximum FMD (%) in Women at Baseline (R^2_{adj} =0.240)

	Beta	Standardized Beta	95% Confidence Interval		p_{adj}
			Lower Limit	Upper Limit	
Constant	23.297	—	20.316	26.278	<0.001
BA diameter (cm)	−52.475	−0.535	−60.566	−44.384	<0.001
RH blood flow (ml/min)	0.008	0.344	0.006	0.011	<0.001
CO (ppm)	−0.036	−0.092	−0.063	−0.008	0.011
HDL-C (mg/dl)	−0.024	−0.069	−0.050	0.001	0.061
Reader	−0.527	−0.101	−0.895	−0.158	0.005

Abbreviations as in Table 1.

Predictors of maximum FMD 1 year after target quit date. Smoking status was available for the 923 (61.4%) subjects who attended the 1-year follow-up visit, of whom 334 (36.2%) successfully quit smoking (abstainers). Subjects who did not return for the 1-year visit were 1 year older than those who returned ($p = 0.032$) and more likely to be male ($p = 0.032$), but did not differ in race ($p = 0.365$) or baseline cigarettes smoked per day ($p = 0.357$). On average, baseline FMD was lower among subjects who did not return (5.8% [SD = 4.5%] vs. 6.5% [SD = 4.2%], $p = 0.005$).

After 1 year, abstainers gained more weight (4.6 [SD = 5.7] kg) than continuing smokers (0.7 [SD = 5.1] kg, $p = 0.007$) and had larger waist circumferences (100.0 [SD = 16.1]cm vs. 96.1 [SD = 15.8] cm, $p < 0.001$). There were no significant differences in change in C-reactive protein ($p = 0.715$) or change in low-density lipoprotein cholesterol (LDL-C) ($p = 0.449$). ΔFMD was similar in men (0.2% [SD = 3.8%]) and women (0.5% [SD = 4.9%], $p = 0.336$). Changes in baseline BA diameter ($p = 0.701$) and FMD ($p = 0.499$) did not differ significantly among treatment arms.

Among subjects who quit smoking, FMD increased from 6.2% (SD = 4.4%) to 7.2% (SD = 4.2%) after 1 year ($p = 0.005$) (Fig. 1). Among subjects who continued smoking, the change in FMD from 6.5% (SD = 4.3%) to 6.6% (SD = 4.1%) was not significant ($p = 0.643$). BA diameter, BA flow, and RH flow did not change among or between abstainers and continuing smokers. Independent predictors of change in FMD after 1 year were abstinence from cigarette smoking ($p = 0.010$), ΔBA diameter ($p < 0.001$), ΔRH flow ($p < 0.001$), ΔLDL-C ($p = 0.004$), and a home smoking ban ($p = 0.04$) ($R^2_{adj} = 0.284$) (Table 6).

Regarding environmental smoke exposure, only the presence of a home smoking ban predicted ΔFMD. Adding the presence of a home smoking ban to the multivariate model attenuated the effect of abstinence on ΔFMD slightly (Online Appendix 3); however, there was no significant interaction between a home smoking ban and smoking cessation ($p = 0.304$). Baseline smoking burden (cigarettes/day) did not affect the change in FMD after cessation; ΔFMD did not differ between quartiles of cigarettes per day, and baseline cigarettes per day was not an independent predictor of ΔFMD ($p = 0.304$) (Online Appendix 4). Other than the home smoking ban, none of the baseline variables, including age and sex, predicted ΔFMD after 1 year. Multivariate models showed no significant associations between ΔFMD and changes in the following variables: C-reactive protein, body mass index, waist circumference, glucose, and HDL-C. BA diameter was a strong predictor of FMD in all models; models of ΔFMD without ΔBA diameter had a much lower R^2_{adj} (0.040).

Discussion

This was the largest prospective, randomized clinical trial to date that evaluated the effects of smoking cessation and continued smoking on endothelial function. Individuals who stopped smoking experienced a significant improvement in endothelial function, despite gaining weight. The cross-sectional and longitudinal components of this study

Table 5 Independent Associations With BA Diameter in Current Smokers at Baseline (Cross-Sectional Analysis, R^2_{adj} = 0.567)

	Beta	Standardized Beta	95% Confidence Interval		p_{adj}
			Lower Limit	Upper Limit	
Constant	0.291	—	0.265	0.317	<0.001
Male sex	−0.089	−0.622	−0.095	−0.083	<0.001
Age (yrs)	0.001	0.133	0.001	0.001	<0.001
Waist circumference (cm)	0.001	0.206	0.001	0.001	<0.001
Glucose (mg/dl)	0.000	0.051	0.000	0.000	0.018
Physical activity (MET-h/day)	0.004	0.067	0.002	0.007	0.001
Reader	−0.002	−0.021	−0.005	0.002	0.304

BA = brachial artery; MET = metabolic equivalent.

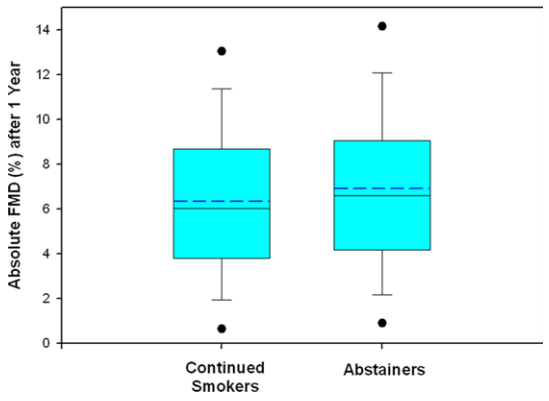

Figure 1 **Absolute FMD Values 1 Year After Quit Date, Expressed as Percentages**

Solid horizontal bars represent means, **dashed horizontal bars** represent medians. **Box margins** are the 25th and 75th percentiles. **Whiskers** are the 10th and 90th percentiles. **Dots** are the 5th and 95th percentiles. FMD = flow-mediated dilation.

highlight important pathophysiological relationships among cigarette smoking, arterial dysfunction, and risk factors for CVD among current smokers and individuals who quit smoking.

The improvement in FMD 1 year after smoking cessation that we identified is consistent with that of a previous report from a smaller, observational cohort study (11). The relationship between abstinence and improved FMD that we identified remained significant even after adjusting for changes in BA diameter and RH blood flow. Although BA diameter and BP did not significantly change with abstinence, heart rate was numerically lower in abstainers compared with continuing smokers, so it is possible that smoking affected vasomotor tone.

A sentinel finding in our study is that FMD improved among abstainers, despite weight gain. Abstainers gained significantly more weight and had larger waist circumferences after 1 year than did continuing smokers, consistent with previous findings (22). Although increases in central adiposity are associated with adverse CVD risk factors and endothelial dysfunction, in regard to FMD, the salutary effects of smoking cessation seemed to outweigh the adverse effects of weight gain. However, weight gain may explain the absence of a significant difference in hsCRP between abstainers and continuing smokers.

The 1% absolute increase in FMD among smokers is not as dramatic as that reported with statins and other interventions (13); however, it is well within the measurement variability of our laboratory (20). In contrast to most statin studies of FMD, our study population was heterogeneous and included individuals with a higher risk factor burden (smoking, overweight, low HDL-C), some of which improved (smoking, HDL-C) and some of which worsened (overweight) over time. Also, our treatment effect may be underestimated because subjects who quit smoking may still be exposed to environmental cigarette smoke. An approximate 1% difference in FMD is associated with a significantly lower rate of incident CVD events (14). Extended follow-up would be necessary to evaluate the relationship between FMD changes 1 year after smoking cessation and future CVD events.

The presence of a home smoking ban was associated with greater improvement in FMD; however, it did not significantly interact with the effect of abstinence on FMD. Therefore, environmental restrictions on smoke exposure and an individual's successful smoking cessation both contribute to improvement in FMD. Further research should include biochemical measures of environmental smoke exposure. Reductions in LDL-C also predicted improvement in FMD. Elevated LDL-C is a risk factor for CVD, and changes in LDL-C are associated with changes in FMD (21). Although smoking status did not influence LDL-C, LDL-C can change over time due to diet, aging, and medication use. Because of our large sample size, we were able to detect changes in FMD related to changes in LDL-C over 1 year. Importantly, baseline markers of smoking intensity and baseline CVD risk factors did not predict changes in FMD after 1 year, suggesting that changes in behaviors associated with smoking and LDL-C influence CVD risk. Mechanistically, endothelial nitric oxide synthase seems to promote release of endothelial progenitor cells from the bone marrow (23). Improved FMD of the BA, as seen in our study, is nitric oxide

Table 6 **Predictors of Change in FMD (%), 1 Year After Target Quit Date (R^2_{adj} = 0.284)**

			95% Confidence Interval		
	Beta	Standardized Beta	Lower Limit	Upper Limit	p_{adj}
Constant	1.237	—	0.033	2.440	0.044
Abstinence status after 1 yr	0.848	0.091	0.207	1.489	0.010
Change in baseline BA diameter (cm)	−107.944	−0.468	−123.951	−91.938	<0.001
Change in RH blood flow (ml/min)	−0.005	−0.277	−0.006	−0.004	<0.001
Change in LDL-C (mg/dl)	−0.020	−0.103	−0.033	−0.006	0.004
Environmental smoke exposure (home smoking ban)	−0.661	−0.073	−1.291	−0.031	0.040
Reader	0.025	0.005	−0.290	0.340	0.877

Abbreviations as in Table 1.

dependent (9,14). It is likely that smoking cessation reduces oxidative damage to endothelial cells, increases nitric oxide availability, and increases mobilization of endothelial progenitor cells from the bone marrow, indicating arterial repair and reduction in CVD risk (4,9,10).

Endothelial dysfunction is one of several mechanisms by which cigarette smoking promotes atherosclerosis (4,5,8,9,11,12). In our cross-sectional analysis of >1,500 individuals who smoked at least 10 cigarettes/day, HDL-C and CO levels significantly predicted FMD, an established measure of endothelial function that predicts future CVD events (9,13,14); however, BA diameter was the most powerful predictor of FMD, accounting for most of the variance in FMD in current smokers. Independent predictors of baseline BA diameter included age, male sex, waist circumference, glucose, and physical activity. These findings are consistent with those of previous reports showing that BA diameter is strongly associated with CVD risk factors and predicts CVD events (14,21). Although BA diameter is the strongest predictor of FMD in smokers, BA diameter seems to reflect the influence of additional CVD risk factors that contribute to endothelial dysfunction and atherogenesis.

We found strong positive correlations among BA diameter, waist circumference, and body mass index, as well as larger BA diameters among men compared with women. FMD in male smokers was predicted by BA diameter and triglycerides, a component of metabolic syndrome. In women, FMD was predicted by BA diameter and low HDL-C, which also is a component of metabolic syndrome and a predictor of CVD. To determine whether BA diameter was masking other significant predictors of FMD, we created separate models without BA diameter and found the expected directional relationships among FMD, age, sex, and CO; however, no other CVD risk factors were independently associated with FMD. These models accounted for less variance than did those that included BA diameter. The confounding relationship between BA size and body size in studies of FMD has been described previously (24,25). Patients with metabolic syndrome have larger BA diameters than those without (25). The inverse relationship between HDL-C and baseline FMD that we observed in our multivariate models most likely was due to intercorrelations between HDL-C, body size (waist circumference, male sex), and BA diameter.

In regard to smoking parameters, CO, a measure of smoking heaviness, was independently associated with baseline FMD, but measures of smoking quantity such as cigarettes per day and pack-years were not. Previous reports had conflicting conclusions about a dose-response relationship between cigarette smoking and CVD risk (4,5). Our study suggests that an objective measure of smoking intensity (e.g., CO) is more likely to yield evidence of a dose-response relationship than are self-reported smoking rates (4,5). This is consistent with evidence that smokers tend to titrate their intake of nicotine by how intensively and efficiently they smoke each cigarette, which is reflected by CO levels, as opposed to the number of cigarettes that they smoke each day (26).

Study limitations. This was a randomized clinical trial of smoking cessation interventions, and thus there were no nonsmoking controls; therefore, we cannot determine the extent to which FMD approached normal values. The stability of FMD among those who continued to smoke, the similar FMD findings in each study arm, and the documented reproducibility of FMD techniques in our laboratory suggest that our findings are not due to chance and would not be seen in nonsmokers (20). Because FMD was only measured at baseline and after 1 year, we could not evaluate the time course of FMD improvement with quitting.

In smoking cessation studies, it is common for subjects who relapse to drop out or miss follow-up visits (27–29). In our study, 38.6% of subjects did not return for their 1-year follow-up FMD visit, which is consistent with the 30% to 43% 1-year dropout rates reported in other recent clinical trials of smoking cessation pharmacotherapy (28,29). Subjects who did not attend the follow-up visit were similar in age, sex, and race to those who did return, and they smoked a similar number of cigarettes/day at baseline. Although their baseline FMD was slightly lower, our findings were robust in many analyses, and it is unlikely that their inclusion would have changed our main finding: that smoking cessation improves endothelial function. Second-hand smoke exposure was not quantified in this study. The presence of second-hand smoke exposure may have led us to underestimate the effect of smoking cessation on FMD. Although significant efforts were made to recruit a racially diverse subject population, nonwhites constituted only about 16% of the study cohort. Differences in the associations between smoking and endothelial function by race may not have been detected. We did not administer nitroglycerin, so an effect of smoking cessation that is not endothelium mediated cannot be excluded. Finally, the long-term effect of smoking cessation on CVD risk was not evaluated; however, data collection for a 3-year analysis of carotid intima-media thickness is ongoing.

Conclusions

In this large, prospective study of current smokers, smoking intensity was independently associated with endothelial dysfunction. One year after smoking cessation, endothelial function improved significantly, despite weight gain. Improvements in endothelial function may mediate some of the reduced CVD risk observed after smoking cessation.

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Key Words: clinical trial ■ endothelial dysfunction ■ risk factors ■ smoking.

APPENDIX

For additional material, please see the online version of this article.